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NATURAL HISTORY OF HTLV III INFECTION IN USAF PERSONNEL:
CLINICAL EVALUATION, LABORATORY EVALUATION,
ASSESSMENT OF IN VIVO AND IN VITRO IMMUNOLOGIC STATUS,
AND DATA STORAGE

FINAL REPORT

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<p>"Natural History of HTLV III Infection in US Air Force Personnel: Clinical Evaluation, Assessment of In Vivo and In Vitro Immunologic Status, and Data Storage" has been a multidisciplinary, multiyear investigation involving a clinical, laboratory and immunological investigation into the natural history, the study of neuropsychiatric and neurological natural history, and the clinical history of transition from asymptomatic HIV infection to symptomatic disease and frank AIDS. During the period of this project, 989 individuals were entered into the WHMC HIV database. Of these, 645 were Walter Reed stage 1; 122 were Walter Reed stage 2; 47 Walter Reed stage 3; 29 Walter Reed stage 4; 47 Walter Reed stage 5; and 43 Walter Reed stage 6 at initial diagnosis. Fifty-six were of uncertain WR stage. Upon re-evaluation, overall rate of progression to higher Walter Reed stage was approximately 15% per year with a lower number in the Walter Reed stage 1 and 2 and a much higher number in Walter Reed stage 3 through 6 advancing yearly. There was a linear correlation with advanced initial Walter Reed stage and likelihood of subsequent</p>					
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progression to Walter Reed stage 6, or frank AIDS. Areas of investigation included: Natural History, Role of putative viral co-factor infections, Neurological and Neuropsychiatric Natural History, Immunology of HIV infections - quantitative and qualitative perturbations.



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1986-1987

During the period of 1986, US Army contract DAMD-17-86-MM-6508 provided funding to WHMC for collection of natural history data and collaboration with extramural researchers in a San Antonio consortium for the purposes of examining concurrent non HIV viral infections as co-factors for progression of disease. This US Army contract enabled Wilford Hall to purchase \$250,000 in laboratory equipment to include: a state of the art \$180,000 cytofluorograph and cell sorter. In addition, \$130,000 in computer software was made available for creating an HIV natural history databank utilizing the Vax mainframe located at Wilford Hall. In addition, two GS-11 immunology laboratory technicians were hired for processing of T lymphocytes cell surface markers for standardized T-helper cell determination and in other specialized projects. In addition, a computer programmer and a data entry clerk were hired as well as a neuropsychiatric technician and an HIV ward secretary. All of these individuals enabled Wilford Hall to cope with an increasing patient workload and to redirect hospital resources back to patient care and away from collection of natural history data. During this first year of DoD force wide screening for HTLV III (subsequently known as HIV 1) approximately 500 active duty personnel were identified as HIV positive. Each individual received a standardized history, physical examination for presence of HIV related morbidity, lumbar puncture for analysis of CSF protein, glucose, cell count, differential, and IgG synthesis and oligoclonal bands.

HIV-1 Culture

In addition, resources were provided to Dr Gordon Dreesman, Ph.D., Chairman of Virology at Southwest Foundation for Biomedical Research for the culture of HIV 1 from infected USAF personnel. This was accomplished with rates of recovery ranging from 40 to 50% for Walter Reed stage 1 and 2 up to 70% for Walter Reed stage 6. In addition, cells and serum were banked at Southwest Foundation for Biomedical Research for future investigation and potential collaborations.

CMV

Another investigation into the role of co-factor viral infection was performed by Dr Kendall Smith, Ph.D., professor of Microbiology at the University of Texas Health Science Center in San Antonio. Dr Kendall Smith in collaboration with Dr Joan Ratner showed that the rate of seropositivity for CMV infection was on the order of 95% in HIV positive patients. This was significantly greater than for an age-matched control population. Subsequently, Dr Smith was able to identify an extremely valuable cohort of approximately 20 HIV+ patients who were CMV seronegative on initial evaluation. These individuals were followed and documented seroconversion was shown to occur in some of these patients. Preliminary insights have subsequently shown that individuals with new CMV seroconversion appear to progress at a significantly higher rate than individuals who remain seronegative at the same initial Walter Reed stage.

Epstein Barr Virus

Similar work by Dr. Ciro Sumaya, M.D., professor of Pediatrics at the University of Texas Health Science Center in San Antonio focused on the relationship between Epstein-Barr virus infection and progression from early to late Walter Reed stage disease in HIV infection. Dr. Sumaya showed similar rates of seropositivity, on the order of 95% for HIV infected patients. In addition, he showed a statistically significantly higher percentage of patients positive for EBV antigen as well as higher titers for EBV viral capsid antibody and subsequently higher numbers of viral DNA copy per infected blood unit in patients with late Walter Reed stage disease compared to early stage disease. In summary, a significant milestone for 1986 through 1987 included the creation of an HIV unit to facilitate clinical research, the hiring of several key personnel who were essential for the continued evaluation and data gathering of HIV infected personnel; and also the creation of a sophisticated data entry collection and analysis system with links to multiple extramural collaborators. As mentioned, virological studies on HIV infection included extramural collaboration with Dr. Gordon Dreesman of Southwest Foundation for Biomedical Research and the potential role of co-factor viral infections was investigated by Dr. Sumaya for EBV and Dr. Smith for CMV.

1987-1988

During the year 1987 through 1988, a total of 768 HIV positive patients were entered into the WHMC databank. Of these, approximately 700 were active duty US Air Force personnel and another 60 were retirees and dependents. At this time, 64 patients met CDC criteria for the diagnosis of AIDS. Of these 768 patients, 59% were Walter Reed 1, of which half had generalized adenopathy of unknown duration; 21% were Walter Reed 2; 6% Walter Reed 3; 3% Walter Reed 4; 4% Walter Reed 5 and 7% Walter Reed 6.

1987 through 1988 was a time of intense clinical data gathering, culminating in scientific presentations at the DoD contract site visits to Wilford Hall in January 1988, the Tri-service HIV meeting in San Antonio later in January 1988, the Society of Air Force Physicians meeting in March 1988, the Tri-service ID meeting at Homestead AFB, FL in March 1988, Walter Reed Institute of Research March 1988 and a presentation by Dr Douglas Marshall (USAF retired) at the WHO working group on HIV neurological disease in Geneva, Switzerland.

A total of 13 abstracts were accepted at the V International Conference on AIDS which was held at Stockholm, Sweden in June 1988. Presentations previously reported to WRAIR and the US Army R&D Command include: correlation of sensorineural hearing loss with HIV infection, HIV viral culture data, detection of viral antigen and quantitative antibody levels in serum and CSF, interval changes in clinical status of CSF by Walter Reed stage, epidemiology and progression of HIV disease by Walter Reed stage, neuropsychiatric functioning in HIV positive patient, CSF findings in asymptomatic individuals infected by HIV, epidemiology of US Air Force force wide screening for HIV, and an investigation of attempted suicide and psychiatric diagnosis in HIV infected patients, an investigation on inhibition of lymphocyte proliferation by HIV Gp120 glycoprotein (soluble suppressor factor) and its association with disease progression, an association of remission of previously diagnosed sarcoidosis, and the utility of gallium 67 scanning in HIV patients with pulmonary disease.

Extramural Collaborations

1987 through 1988 was a very productive year for new extramural collaborations. The most important of these was the collaboration Dr Gene Shearer, a cellular immunologist at the National Cancer Institute who in collaboration with Dr Hanna Golding and Dr Jay Berzofsky of the NCI intensively evaluated the T helper cell functional capabilities of our HIV infected individuals and with us correlated these with clinical, immunological and his orical data.

Summary

In summary, 1987 through 1988 was a extremely active year for clinical observation and marked the beginning of intense investigation into HIV neurological disease, neuropsychiatric disease and qualitative evaluation of immunological aborations and correlations with quantitative T cell depletion.

1988-1989

During the time period of September 1988 through September 1989 a total of 878 HIV positive patients were entered into the WHMC database. During the 1989 calendar year investigation into the natural history of HIV infection continued. In addition, intensive laboratory correlation occurred in the search for surrogate markers of disease progression which would predict at an earlier stage those who are at highest risk for progression to Walter Reed stage 6 or AIDS. Among these parameters evaluated include absolute CD4A helper count, CD4 to CD8 ration, serum IgG, IgA, IgM, IgE, presence of partial or complete anergy, presence of oral hairy leukoplakia, as well as standard chemistry profiles and hemograms. All of the above parameters correlated significantly in 1 fashion or another with presence of advanced disease at presentation but upon multivariant analysis, only low CD4A helper cell count and serum IgA were independent predictors of disease progression.

During 1989, collaboration with investigators at the National Cancer Institute including Dr Gene Shearer, Dr Berzofsky and Dr Golding included analysis of T helper cell responses to mitogens and specific soluble antigens, analysis and responsiveness to T lymphocyte IL-2 production and in vitro proliferation in response to synthetic HIV peptides generated by Dr Berzofsky. In addition, Dr Golding analyzed antibodies which cross react with HLA DR and HIV 1 gp41 which was present in statistically significantly higher levels in individuals at later Walter Reed stage disease than those with early stage disease supporting the hypothesis that there may be an autoimmune reaction between HLA DR and HIV gp41 leading to progressive T cell depletion and functional impairment of T lymphocyte responses and progressive immunodeficiency.

Neurological Natural History

The HIV neurological natural history investigation reached its peak of productivity during calendar year 1989. All patients received a complete neurological evaluation, a lumbar puncture and CSF analysis with the parameters previously described. In selected cases, myelin basic protein, HIV culture and p24 antigen detection was performed on CSF. Among the neurological findings of note, we found a low prevalence of CNS opportunistic infection as presenting symptom of AIDS. In addition, HIV or AIDS related dementia did not occur in early stage patients. In a study of neurologically asymptomatic HIV 1 infected Air Force personnel with at least two CSF studies, and a minimum of 270 days between analysis it was found that there were significant increases over time in nucleated cell counts, measures of interthecal IgG production and decrease in protein levels. When CD4A counts dropped below 400 per cubic millimeter, cellular responses and immunological changes were less impressive. These findings suggest that CSF immune response is maximal when patients are immunocompetent and more blunted as they become T cell depleted. Neuropsychiatric testing showed absence of dementia at early stages although there was the appearance of considerable test related anxiety for individuals with a first time diagnosis of HIV infection.

Two other neurologically related studies were accomplished including assaying for the presence of anticardiolipin and IgG antibodies in the CSF which showed that in patients with serum anticardiolipin antibodies there was no statistically significant difference between patients and controls for CSF anticardiolipin antibody IgM but 9 of 12 had abnormal CSF anticardiolipin IgG levels.

A study of neurological nerve conduction velocities on nearly 300 HIV infected patients showed a statistically significantly slower rate of conduction with T cell counts of less than 200/cubic millimeter.

Oral Hairy Leukoplakia

During this year, a controlled cohort of patients with oral hairy leukoplakia was identified of which 72 were confirmed by biopsy. The overall prevalence of biopsy confirmed OHL in the entire cohort is approximately 9%.

Interestingly, whites were disproportionately represented in the OHL+ group, even after controlling for possible selection bias, based on clinical OHL cases who refused tongue biopsy. An OHL+ cohort was compared to an OHL- cohort of 495 HIV+ patients. Patients who had OHL pathologically diagnosed with three times more likely to subsequently develop more severe clinical findings, either by advancing beyond Walter Reed stage 3 with an odds ratio of 2.9 at p value of less than .001 or beyond Walter Reed stage 4 with an odds ratio of 3.5 with a p value of less than .001. Similarly, they were also 3.3 times more likely to drop below 200 absolute CD4a cells per cubic millimeter, with an odds ratio of 3.3 and a p value of less than 0.001. Progression to CD4 of less than 200 or beyond Walter Reed stage 3 persisted even when controlling for initial CD4a, race or age. P was less than 0.01. Although OHL+ patients had the same mean age, they had an initial CD4a count of 550 CD4 cells per cubic millimeter which was significantly different from the mean of 650 in the OHL- group. Again, despite changes in initial CD4a level when these were controlled for, there was significantly more rapid fall in helper cell in the OHL+ than in OHL- group.

Rapid Progressors

We have identified a cohort of 26 rapid progressors and matched them with a control population of 26 patients who did not progress for: CD4 initial T-cell count, race, age and sex. Laboratory markers which distinguished progressors from non-progressors included serum LDH, which was higher in progressors at initial admission, $p < 0.02$, serum IgA concentration, $p < 0.001$, initial IgE values, hemoglobin and platelet count. Syphilis and hepatitis B serologies were not predictive of progression. The change in serum IgE during the interval trended toward significance at $p < 0.06$. In addition, very recently we have begun performing in-house assays for serum neopterin, TNF/cachetin and beta-2 microglobulin which in other cohort studies have been independent predictors of disease progression.

Although numbers were small, it appears that this partially confirms the observations of Greenspan et al (JID 1987, 155, pg 475) who report OHL as a significant bad prognostic marker for progression to AIDS over a 30 month period.

Summary

Milestones for 1988-1989 included: extension and consolidation of observations obtained in 1987 and 1988 on the natural history of HIV-1 infection, role of oral hairy leukoplakia, rates of progression of their natural history study patients, further followup on patients with HIV neurological evaluation and additional studies on subgroups of rapid versus slow progressors in the overall HIV+ patient population.

Because funding for 1988 through 1989 was piecemeal, the major efforts during this time period were directed at insuring continued evaluation of existing projects.

The cumulative number of HIV+ patients in the databank reached 989. Over the last 6 months, we have performed 236 evaluations including 59 new patients and 177 re-evaluations.

Demographic characteristics in our cohort have tended to follow the same patterns as previously established with approximately with 50% caucasians, 40% blacks and 10% other racial and ethnic groups.

Close interactions among multidisciplinary groups in comparing natural history data, CD4 cell counts, neuropsychiatric testing, neurological findings, pediatric HIV infections, spouse studies and many other US Army RV protocols were reviewed, accepted and principal investigators designated at Wilford Hall USAF Medical Center.

1989 was largely a year of transition for the HIV project at WHMC as US Army funding mechanisms changed but monies were made available to ensure continuity of the project until such time as the Henry M. Jackson Foundation memorandum of understanding was completed between the US Air Force, US Army and all other principals.

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91. Rundell J, Praus D, Thomason J, Brown G, Marshall D, Holloway H, Boswell R. CSF Findings and Request for Psychiatric Examinations in HIV-Infected Patients. Presented at the Neuropsych Conference VI International Conference on AIDS, Monterey, California, June 1990.
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